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Research Article

Phytochemical Properties of Different Plant Parts of *Eucalyptus camaldulensis*

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ABSTRACT

This study presents a comparative analysis of soil properties and phytochemical constituents of *Eucalyptus camaldulensis* from two locations: Daphar I.P. and Mitha Tiwana I.P. Soil samples were analyzed for physical and chemical parameters, while phytochemical analyses were conducted on leaves, stem bark, and seeds using water, ethanol, and methanol extracts. The soils at both sites were classified as loam, with similar texture but varying chemical characteristics. Slightly alkaline pH values (7.96 and 7.93) were recorded at both sites, which may limit the availability of phosphorus and micronutrients. Electrical conductivity (EC) was significantly higher at Daphar (3.506 mS/cm) compared to Mitha Tiwana (0.976 mS/cm), indicating slight salinity at the former. Organic matter was high at both sites, supporting plant metabolic processes. Available phosphorus was low at both locations, suggesting a deficiency that could hinder plant growth and secondary metabolism. Potassium levels were moderate, supporting essential physiological functions. Phytochemical screening revealed site- and solvent-dependent variation. Alkaloids were present only in methanol and ethanol extracts from Daphar, while tannins and saponins were widely detected, especially in leaf and stem bark extracts. Phenols were absent across all samples, likely due to the absence of environmental stress. Flavonoids were limited to methanol-extracted seeds, and steroids were detected in water and ethanol extracts of various plant parts. Terpenoids were absent in all samples. In conclusion, both soil fertility and environmental conditions appear to influence the phytochemical profile of *E. camaldulensis*, with Daphar showing greater diversity in bioactive compounds, potentially due to higher EC and organic matter content.

Keywords: *Eucalyptus camaldulensis, Botanical parts, Phytochemical screening, Extracts, Edaphic properties, Medicinal Value.*

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Introduction

Technical terms such as phytochemicals, biogenic volatile organic compounds (BVOCs), secondary compounds, secondary products, and natural products may initially appear distinct, but they all refer to a common group of organic molecules known as secondary metabolites in plants. These metabolites are not essential for primary physiological processes such as growth, development, cell structure, or maintenance, and thus do not cause immediate death if absent. However, they play a crucial role in plant defense mechanisms against herbivores and pathogens, thereby contributing to the long-term survivability of plants (Weisshaar and Jenkins, 1998).

The study of secondary metabolites for pharmaceutical applications has gained significant attention based on strong epidemiological evidence suggesting that plant-based diets are associated with a reduced risk of cardiovascular diseases and cancer. Approximately 80% of rural populations rely on traditional medicine for primary healthcare, much of which is based on plant extracts (Anurag et al., 2014). In agriculture, secondary metabolites are equally important, having been historically used and currently researched for their insecticidal properties. Examples include azadirachtin from *Azadirachta indica* (neem), nicotine from *Nicotiana* spp., pyrethrins from *Pyrethrum* spp., piperine from *Piper* spp., and rotenone from *Derris* spp. (Khater, 2012).

Eucalyptus camaldulensis (EC) has a long-standing history of medicinal use. It has been employed for the treatment of colds and influenza and is widely recognized for its antiseptic, analgesic, and antipyretic properties (El-Sayed, 2012). Traditional uses include treatment of respiratory conditions such as bronchitis and croup, and its dried leaves have been smoked to relieve asthma. *E. camaldulensis* has also been used for fevers associated with malaria, typhoid, and cholera, as well as for wound healing and treatment of burns and ulcers (Sani et al., 2014). Moreover, *E. camaldulensis* is used to treat sore throats and infections of the respiratory and urinary tracts. The essential and volatile oils from the plant serve as expectorants and are employed in the treatment of lung diseases (Ayepola and Adeniyi, 2008).

The biosynthesis of secondary metabolites in nature is influenced by a variety of factors, leading to considerable variability in their composition and quantity. These factors include physiological differences (e.g., organ development, pollinator cycles, and type of plant tissue), environmental conditions (e.g., climate, pollution, pests, and diseases), edaphic factors (e.g., soil composition and drainage), geographic location, genetic diversity, as well as storage, social, and political factors (Reshi et al., 2232). Conventional separation techniques using various solvent systems have been developed to extract and identify secondary metabolites (Jan et al., 2021).

Phytochemical screening of methanol-extracted leaves of *E. camaldulensis* revealed the presence of saponins, cardiac glycosides, and tannins, whereas alkaloids and anthraquinones were absent (Ayepola and Adeniyi, 2008). Another study found tannins to be abundant in leaves, stem bark, and seeds using both aqueous and ethanolic extracts. Saponins were detected in aqueous extracts of leaves and stem bark but were absent in seeds. Alkaloids were found only in ethanolic extracts across all plant parts, while flavonoids were absent in both types of extracts (Sani et al., 2014). Aqueous extracts of *E. camaldulensis* leaves revealed the presence of saponins and alkaloids, while flavonoids and tannins were absent. Conversely, ethanolic extracts of the leaves showed the presence of flavonoids only (Sani et al., 2014). Methanol-extracted leaves and stem bark also demonstrated the presence of glycosides, saponins, and tannins (Alghamdi and Ababutain, 2019; Etemadi et al., 2020; Adawara et al., 2021; Usman et al., 2021; Deshi et al., 2023; Hussein et al., 2023). Phytochemical analysis of eucalyptus oil further confirmed the presence of phytosterols, phenols, tannins, and flavonoids (Neelam et al., 2014).

Numerous researchers have identified soil structure, nutrient concentration, and other edaphic factors as major influences on the composition and yield of secondary metabolites, particularly volatile compounds. For example, despite the absence of morphological variation in trichome indumenta, significant differences were observed in essential oil composition among *Thymus caespititius* populations growing just 200 m apart, suggesting a role for genetic and edaphic factors (Pereira et al., 2009). Poorly drained soils negatively affect plant growth, survival, and essential oil yield. Although soil supplementation with nitrogen, phosphorus, and potassium generally increases oil yield, the individual contribution of each nutrient can have variable effects on oil composition. In some cases, nitrogen concentration was found to have no effect on oil composition despite affecting yield (Figueiredo et al., 2008).

The objectives of the present study were to assess the presence and diversity of secondary metabolites in various botanical parts of *E. camaldulensis* and to explore their potential uses; to evaluate the influence of environmental factors, particularly soil properties, on the production of secondary metabolites in different plant parts; and to determine the qualitative response of different botanical parts to various extracting solvents.

Materials and Methods

Soil and plant sample collection

Soil samples were collected from three different sites within each locality at three distinct depths: 0-6 inches (approximately 0-20 cm), 6-12 inches (approximately 20-40 cm), and 12-18 inches (approximately 40-60 cm). Each sample was labeled with the collection date and location and transported to the laboratory for analysis of physical and chemical characteristics, particularly in relation to BVOC (biogenic volatile organic compound) production. Plant samples, including leaves and stem bark, were collected from six different trees in each locality. Seeds were obtained from corresponding nursery plantations. All plant samples were labeled with date, time, and location, and stored at room temperature for further analysis.

Soil sample preparation and plant extraction

The three soil cores from each depth per site were homogenized to prepare a composite sample for laboratory analysis. Leaf, stem bark, and seed samples were air-dried in the shade, followed by oven-drying at 80°C for

approximately three hours to remove moisture. The dried plant material was ground into a fine powder using an electric grinder. Twelve samples (leaves and stem bark) and two seed samples from the two localities were prepared for solvent extraction. Two grams of each powdered plant part were separately dissolved in three solvents: distilled water, methanol, and ethanol. The mixtures were incubated overnight at room temperature. After incubation, the samples were filtered using Whatman filter paper, and the filtrates were used for phytochemical screening to identify secondary metabolites.

Analysis of soil and plant samples

Determination of soil pH

Soil pH was determined by preparing a suspension of air-dried soil and distilled water in a 1:2.5 (soil:water) ratio. Ten grams of soil were mixed with 25 ml distilled water, stirred intermittently for 15-20 minutes, and then measured using a calibrated pH meter (Ryan et al., 1977).

Determination of electrical conductivity (EC)

Electrical conductivity was measured using a conductivity meter with the same soil suspension used for pH measurement. The suspension was stirred twice at 30-min intervals for 10 min each time. The meter was calibrated using a saturated solution of calcium sulfate dihydrate (Chapman and Pratt, 1978).

Determination of organic matter

Organic matter content was estimated using the Walkley-Black method (Walkley and Black, 1934), and calculated as:

% Organic Matter = (% Organic Carbon) / 0.58 = % C × 1.72

Where 0.58 and 1.72 are standard correction factors.

Determination of available phosphorus

Available phosphorus was determined using the Micro-Vanadate-Molybdate method (Olsen et al., 1954). The phosphorus concentration was calculated using the following formula:

P (mg/100 g) = (Reading × Factor / 10) × (100 / Dry weight at 105 °C)

Determination of available potassium

Available potassium was extracted using the ammonium acetate method and quantified using a flame photometer. The slope extraction method was used for calculating potassium concentrations in the samples.

Determination of soil texture

Soil texture was determined using the USDA textural triangle based on particle size distribution.

Phytochemical screening

Test for alkaloids

Mayer's reagent was used to detect alkaloids. The appearance of a white or cream precipitate indicated their presence.

Test for terpenoids

Samples were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform, followed by the slow addition of concentrated sulfuric acid. The formation of a reddish-brown interface indicated the presence of terpenoids.

Test for flavonoids and flavones

Samples were treated with dilute sodium hydroxide followed by dilute hydrochloric acid. A yellow coloration with NaOH that turned colorless upon HCl addition indicated the presence of flavonoids and flavones.

Test for tannins

Methanol, ethanol, and aqueous extracts (0.5 ml each) were treated with 1 ml of distilled water and 1-2 drops of ferric chloride solution. A blue coloration indicated gallic tannins, while green-black indicated catecholic tannins.

Test for phenols

One milliliter of each extract was mixed with 1 ml of distilled water or ethanol, followed by the addition of a few drops of ferric chloride solution. The appearance of red, blue, green, or purple coloration confirmed the presence of phenolic compounds (Ferric Chloride Test).

Test for saponins

Two milliliters of each extract were mixed with 2 mlL of distilled water and vigorously shaken in a test tube. The formation of a stable 1 cm layer of foam indicated the presence of saponins (Foam Test).

Results and Discussion

Soil analysis

The results of the soil analyses from the two locations, Daphar I.P. and Mitha Tiwana I.P., are presented in Table 1 and Figure 1, respectively. The physical and chemical properties of soil are influenced by several factors, including parent material, climate, topography, biological activity, and time, which collectively contribute to soil formation (Brady and Weil, 2002).

Table 1. Physical and chemical properties of soils from Daphar and Mitha Tiwana industrial plantations.

Parameter	Daphar I.P. (Mean ± SD)	Mitha Tiwana I.P. (Mean ± SD)	Remarks
Soil Texture	Loam (Silt 40%, Sand 35%, Clay 25%)	Loam (Silt 40%, Sand 40%, Clay 20%)	Same texture at both sites based on USDA classification
pH	7.96 ± 0.12	7.93 ± 0.05	Slightly alkaline; affects availability of P, Zn, Fe, Mn, and B
Electrical Conductivity	3.506 ± 0.095 mS/cm	0.976 ± 0.061 mS/cm	Daphar: Slightly saline; Mitha Tiwana: Non-saline
Organic Matter (%)	4.8 ± 1.9	4.1 ± 1.9	High OM may enhance soil temperature and influence secondary metabolism
Available P (mg/kg)	3.46 ± 0.75	3.8 ± 1.03	Both sites deficient; limits plant growth and secondary metabolism
Available K (mg/kg)	128 ± 11.31	132.66 ± 12.25	Moderate fertility; supports plant metabolic activities

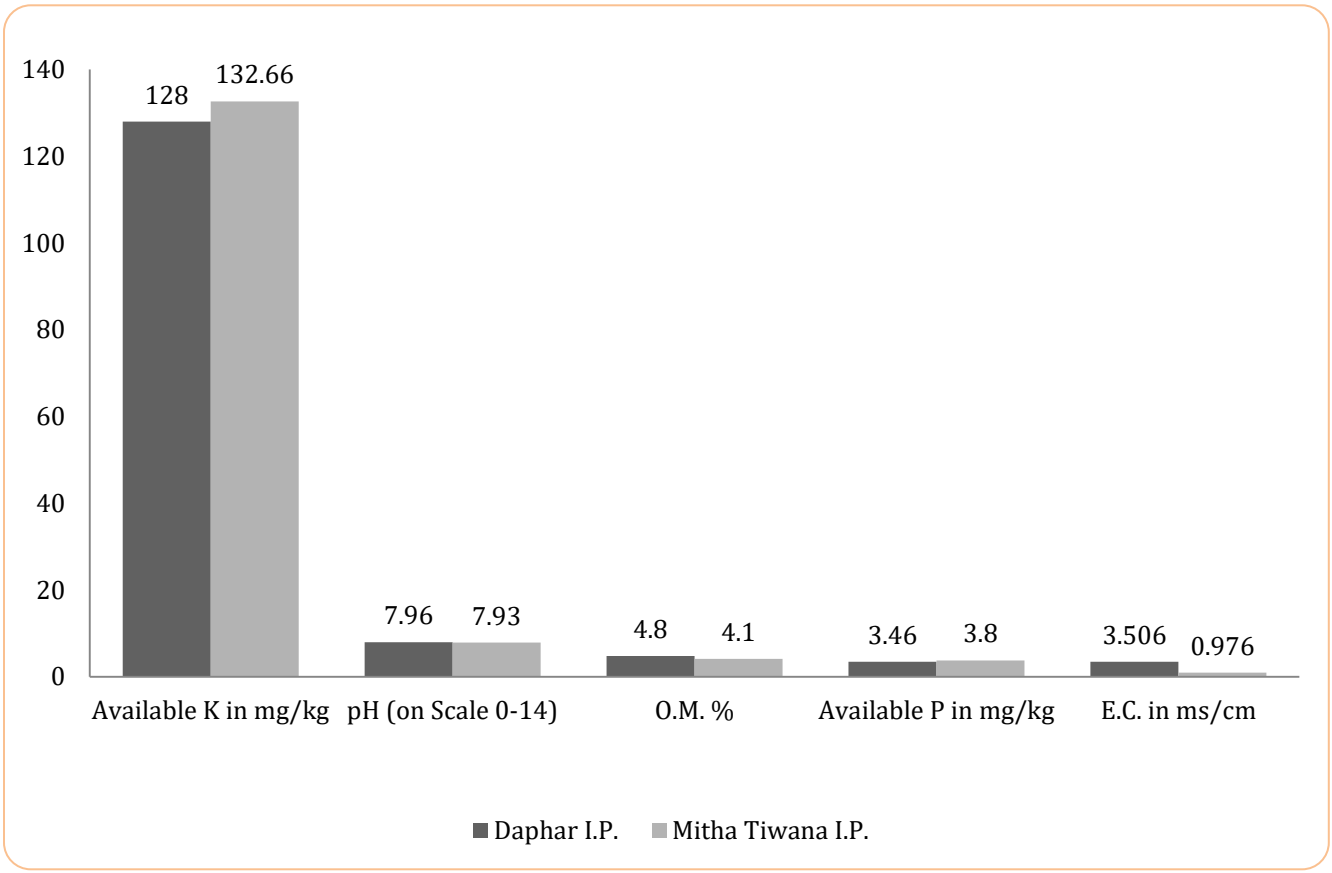

Figure 1. Comparative soil analysis of Daphar and Mitha Tiwana I.P. sites.

The analysis revealed variations between the two sites in parameters such as pH, electrical conductivity (EC), organic matter (O.M.) content, available phosphorus, and potassium levels. However, soil texture was consistent across both sites. Using the USDA textural triangle and confirmed by the saturation percentage method, the soil texture at both locations was classified as loam. The relative proportions of silt, sand, and clay were 40%:35%:25% for Daphar and 40%:40%:20% for Mitha Tiwana.

The soil pH at Daphar and Mitha Tiwana was 7.96 ± 0.12 and 7.93 ± 0.05, respectively, indicating slightly alkaline conditions at both sites. At this pH, most nutrients are adequately available, except phosphorus among macronutrients and micronutrients such as zinc, iron, manganese, and boron, which are optimally available at pH ranges of 6-7 and 5-7, respectively. Soil pH is a critical factor influencing nutrient availability and overall plant metabolism (Khaliq et al., 2021).

The EC was recorded as 3.506 ± 0.095 mS/cm for Daphar and 0.976 ± 0.061 mS/cm for Mitha Tiwana. This indicates that the former site exhibits slight salinity, whereas the latter is non-saline. Nonetheless, both EC levels are within the safe range for plant secondary metabolism.

The organic matter content was relatively high at both sites, measured as 4.8% ± 1.9% at Daphar and 4.1% ± 1.9% at Mitha Tiwana. This elevated organic matter may be attributed to higher precipitation and elevated temperatures during the study period. Soil organic matter plays a vital role in enhancing soil temperature by increasing its heat absorption capacity, which can influence plant secondary metabolic processes.

Available phosphorus levels were 3.46 ± 0.75 mg/kg at Daphar and 3.8 ± 1.03 mg/kg at Mitha Tiwana, indicating phosphorus deficiency at both sites. As phosphorus is one of the three primary macronutrients, its deficiency negatively affects plant growth and impairs secondary metabolic pathways in species such as *E. camaldulensis*.

Available potassium was found to be 128 ± 11.31 mg/kg at Daphar and 132.66 ± 12.25 mg/kg at Mitha Tiwana. These values suggest moderate fertility regarding potassium, another essential macronutrient, thereby supporting favorable conditions for plant metabolic activities.

Phytochemical Analysis

The phytochemical analysis was conducted on different plant parts, leaves, stem bark, and seeds, extracted using three solvents: water, ethanol, and methanol. The analysis employed standard reagents and methods, as outlined in the previous chapter. The characteristic color changes or reactions were used as indicators of the presence or absence of specific phytochemicals. The results for Daphar and Mitha Tiwana I.P. are summarized in Tables 2 to 7.

Table 2. Phytochemical components of *E. camaldulensis* plant parts in aqueous extracts from Daphar I.P.

Botanical Part	Phytochemicals					
	Alkaloids	Tannins	Phenols	Saponins	Flavonoids/ Flavones	Steroids/ Terpenoids
Leaves	6 A	5 P	6 A	6 A	6 A	6 P/6 A
Stem/bark	6 A	5 P	6 A	6 A	6 A	6 P/6 A
Seeds	A	P	A	A	A	P/A

*P and A refers to phyto-chemical found or missing in the sample

**Number refers to samples in which respective phyto-chemical was present or absent.

Table 3. Phytochemical components of *E. camaldulensis* plant parts in Ethanol extracts from Daphar I.P.

Botanical Part	Phytochemicals					
	Alkaloids	Tannins	Phenols	Saponins	Flavonoids/ Flavones	Steroids/ Terpenoids
Leaves	6 P	5 P	6 A	4 P	6 A	5 P/A
Stem/bark	6 P	5 P	6 A	3 P	6 A	5 P/A
Seeds	P	P	A	P	A	A/A

*P and A refers to phyto-chemical found or missing in the sample

**Number refers to samples in which respective phyto-chemical was present or absent.

Table 4. Phytochemical components of *E. camaldulensis* plant parts in Methanol extracts from Daphar I.P.

Botanical Part	Phytochemicals					
	Alkaloids	Tannins	Phenols	Saponins	Flavonoids/ Flavones	Steroids/ Terpenoids
Leaves	6 P	5 P	6 A	4 P	6 A	6 A/A
Stem/bark	6 P	5 P	6 A	3 P	6 A	6 A/A
Seeds	P	P	A	P	A	P/A

*P and A refers to phyto-chemical found or missing in the sample
**Number refers to samples in which respective phyto-chemical was present or absent.

Table 5. Phytochemical components of *E. camaldulensis* plant parts in aqueous extracts from Mitha Tiwana I.P.

Botanical Part	Phytochemicals					
	Alkaloids	Tannins	Phenols	Saponins	Flavonoids/ Flavones	Steroids/ Terpenoids
Leaves	6 A	4 P	6 A	6 A	6 A	5 P/6 A
Stem/bark	6 A	4 P	6 A	6 A	6 A	5 P/6 A
Seeds	A	A	A	A	A	P/A

*P and A refers to phyto-chemical found or missing in the sample
**Number refers to samples in which respective phyto-chemical was present or absent.

Table 6. Phytochemical components of *E. camaldulensis* plant parts in Ethanol extracts from Daphar I.P.

Botanical Part	Phytochemicals					
	Alkaloids	Tannins	Phenols	Saponins	Flavonoids/ Flavones	Steroids/ Terpenoids
Leaves	6 A	4 P	6 A	3 P	6 A	4 P/A
Stem/bark	6 A	4 P	6 A	2 P	6 A	4 P/A
Seeds	A	P	A	P	A	A/A

*P and A refers to phyto-chemical found or missing in the sample
**Number refers to samples in which respective phyto-chemical was present or absent.

Table 7. Phytochemical components of *E. camaldulensis* plant parts in Methanol extracts from Daphar I.P.

Botanical Part	Phytochemicals					
	Alkaloids	Tannins	Phenols	Saponins	Flavonoids/ Flavones	Steroids/ Terpenoids
Leaves	6 A	4 P	6 A	3 P	6 A	6 A/A
Stem/bark	6 A	4 P	6 A	2 P	6 A	6 A/A
seeds	A	P	A	P	A	A/A

*P and A refers to phyto-chemical found or missing in the sample
**Number refers to samples in which respective phyto-chemical was present or absent.

Alkaloids were absent in the aqueous extracts of all plant parts at both locations but were present in the methanol and ethanol extracts of all plant parts collected from Daphar. In contrast, methanol and ethanol extracts of samples from Mitha Tiwana did not show the presence of alkaloids. Alkaloids are known for their insecticidal properties, as in nicotine sulfate (Jabeen et al., 2022).

Tannins were detected in all plant parts extracted with any of the three solvents in five samples from Daphar and four samples from Mitha Tiwana. Tannins exhibit antimicrobial and medicinal properties and contribute to physiological activity in plants. Saponins were present in ethanol and methanol extracts of leaves and stem bark in four and three samples from Daphar and in three and two samples from Mitha Tiwana, respectively. Saponins are known for their expectorant, emulsifying, and antifungal properties.

Phenols were absent in all samples from both locations, which may be attributed to the absence of water stress during the study period. Similarly, flavonoids/flavones were detected only in methanol-extracted seeds at both locations. The absence of these compounds in other parts may be due to the lack of drought conditions, as flavonoids often accumulate in response to environmental stress.

Steroids were detected in water and ethanol extracts of leaves and stem bark but were absent in the methanol extracts of these tissues. Steroids were present in water and ethanol extracts of seeds, but absent in the methanol extracts at both sites. Terpenoids were entirely absent in all samples. The comparisons of phytochemicals in both sites are given in Figures 3 to 5.

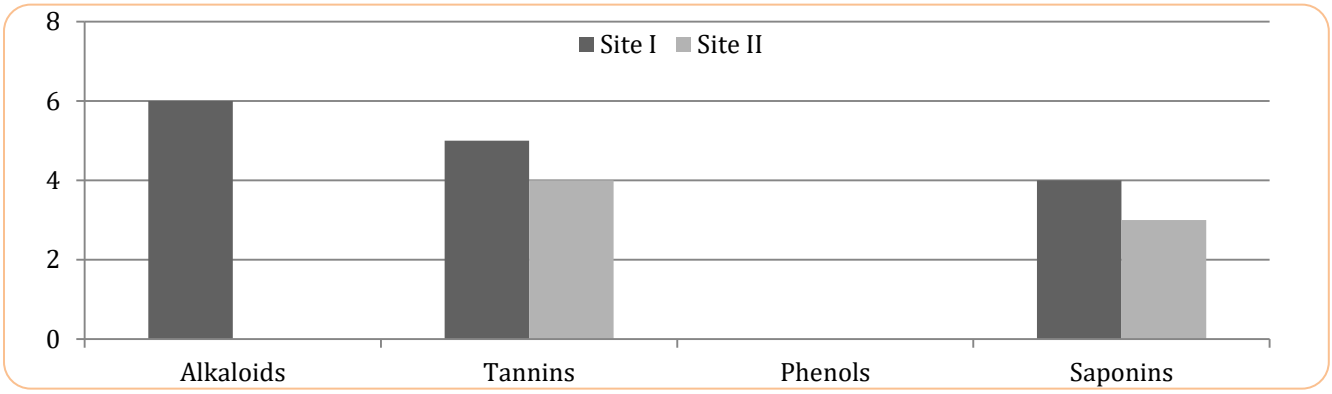

Figure 2. Phytochemicals detected in methanol-extracted leaf samples from Site I and Site II (vertical axis indicates number of positive samples).

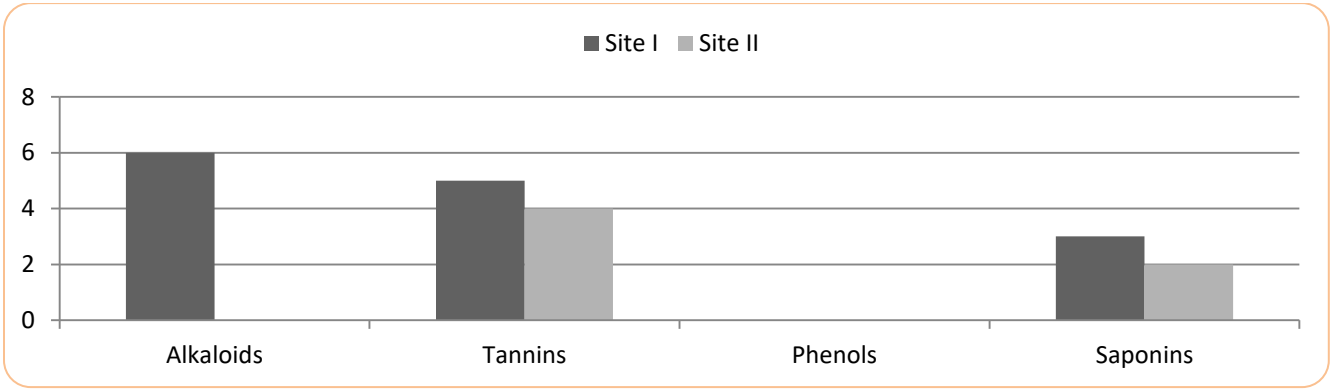

Figure 3. Phytochemicals detected in methanol-extracted stem bark samples from Site I (Daphar) and Site II (Mitha Tiwana). (Vertical axis indicates the number of positive samples.).

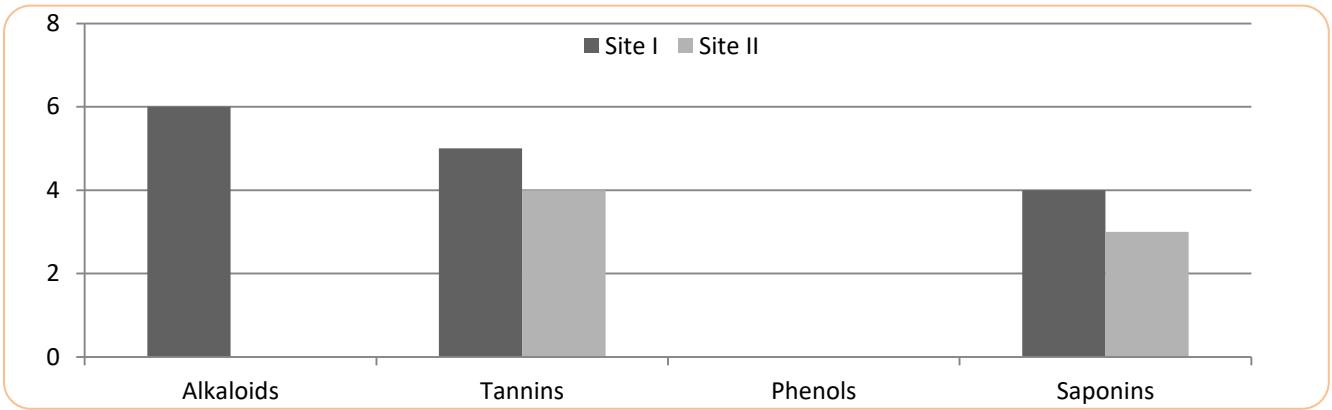

Figure 4. Phytochemicals Detected in Ethanol-Extracted Leaf Samples from Sites I and II (Vertical Axis Represents Number of Samples).

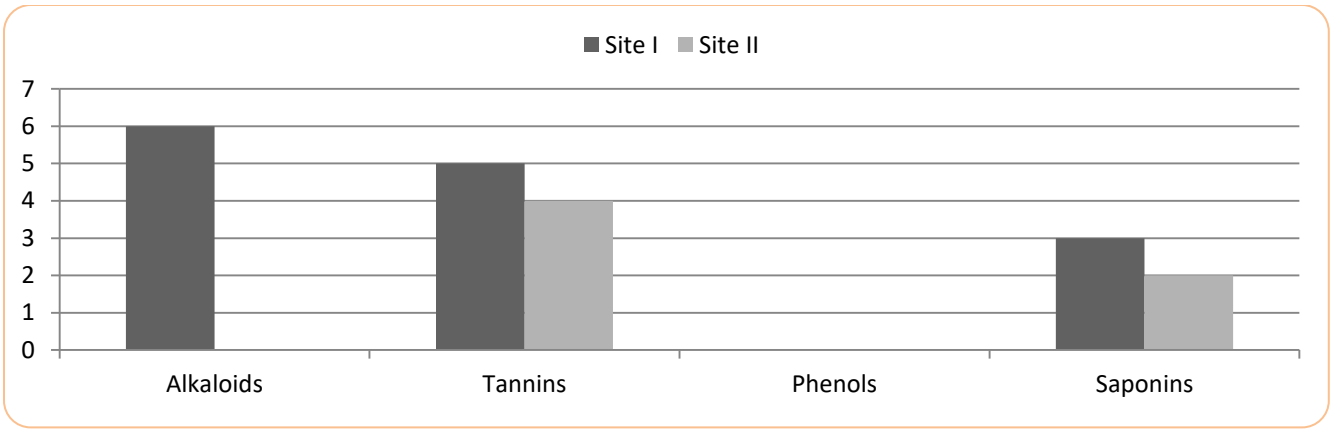

Figure 5. Number of ethanol-extracted stem bark samples yielding phytochemicals at Sites I and II (vertical axis indicates sample count).

These results are consistent with previous studies (Figueiredo et al., 2008; Sani et al., 2014; Alghamdi and Ababutain, 2019; Etemadi et al., 2020; Mohammadi Bazargani et al., 2021; Hussein et al., 2023).

Statistical analysis revealed no significant association between edaphic factors and phytochemical production. The minor differences observed between the two locations may be attributed to geographic, genetic, or environmental variability (Riahi et al., 2015; Aman et al., 1985).

Overall, the presence of a specific phytochemical in one plant part generally corresponded to its presence in other parts at both sites. The types of phytochemicals identified were consistent across both localities, indicating no strong relationship between edaphic conditions and the qualitative nature of BVOCs. Methanol extracts yielded the most diverse range of secondary metabolites, followed by ethanol, with aqueous extracts being the least effective.

Conclusion

The results revealed that secondary metabolites such as tannins, saponins, and steroids were consistently present in all plant parts examined, whereas the presence of alkaloids, flavonoids, and terpenoids varied among different plant parts. Notably, phenols were absent in all samples. The phytochemicals detected at Site I (Daphar) were also present at Site II (Mitha Tiwana), indicating consistency across locations. Statistical analysis showed no significant association between soil characteristics and the type of phytochemicals produced by the plant. Among the solvents used for extraction, methanol proved to be the most effective in extracting a wider range of phytochemicals, followed by ethanol, with water being the least effective.

Authors' Contributions

MH and LA conceptualized, designed the study, developed the methodology, collected specimens and performed phytochemical analyses, MH collected, arranged and analyzed the data; LA supervised the work; MH drafted the manuscript; LA proofread and approved the final version.

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This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Addressed

SDG 3: Good Health and Well-being
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

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